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► To cite this version:

Valentina Baldazzi, Pierre Valsesia, Michel Génard, Nadia Bertin. Organ-wide and ploidy-dependent regulations both contribute to cell size determination: evidence from a computational model of tomato fruit. 2018. hal-01953178

HAL Id: hal-01953178

<https://inria.hal.science/hal-01953178>

Preprint submitted on 12 Dec 2018

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**1Organ-wide and ploidy-dependent regulations both contribute to cell size
2determination: evidence from a computational model of tomato fruit**

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9

10

11Abstract

12

13The development of a new organ is the result of coordinated events of cell
14division and expansion, in strong interaction with each other. This paper
15presents a dynamic model of tomato fruit development that includes cells
16division, endoreduplication and expansion processes. The model is used to
17investigate the interaction among these developmental processes, in the
18perspective of a neo-cellular theory. In particular, different control schemes
19(either cell-autonomous or organ-controlled) are tested and results
20compared to observed data from two contrasted genotypes. The model
21shows that a pure cell-autonomous control fails to reproduce the observed
22cell size distribution, and an organ-wide control is required in order to get
23realistic cell sizes. The model also supports the role of endoreduplication
24as an important determinant of the final cell size and suggests a possible
25interaction through carbon allocation and metabolism.

26

27**Keywords:** cell size, division, development, expansion, endoreduplication,
28growth, model, ploidy, tomato

29

30INTRODUCTION

31 Understanding the mechanisms underpinning fruit development from its
32early stages is of primary importance for biology and agronomy. Indeed, early
33stages are highly sensitive to biotic and abiotic stresses, with important

34consequences on fruit set and yield. The development of a new organ is the
35result of coordinated events of cell division and expansion. Fruit growth starts
36immediately after pollination with intensive cell division. As development
37proceeds, the proliferative activity of cells progressively slows down giving way to
38a phase of pure cell enlargement during fruit growth and ripening. In many
39species, including tomato, the transition from cell division to expansion phases is
40accompanied by repeated DNA duplications without mitosis, a process called
41endoreduplication. The exact role of endoreduplication is still unclear. A strong
42correlation between cell ploidy (i.e number of DNA copies) and final cell size has
43been observed in different species (Bertin, 2005; Lee et al., 2007; Melaragno et
44al., 1993; Rewers et al., 2009), suggesting a role of endoreduplication into the
45control of organ growth (Breuer et al., 2010; Chevalier et al., 2011).

46 Understanding the way cell division, endoreduplication and expansion
47processes interact is crucial to predict the emergence of important morphological
48traits (fruit size, mass, shape and texture) and their dependence on
49environmental and genetic factors. Historically, a big debate has opposed two
50contrasting views, the cellular vs the organismal theory that set the control of
51organ growth at the level of the individual cell or of the whole tissue, respectively
52(reviewed in (Beemster et al., 2003; Fleming, 2006; John and Qi, 2008). In the
53recent years, a consensus view, the neo-cellular theory, has eventually emerged.
54Accordingly, although individual cells are the units of plant morphology, their
55behavior (division, expansion) is not autonomous, but coordinated at the organ
56level by cell-to-cell communication mechanisms, thus creating an effective
57interaction between cellular and whole-organ behavior (Beemster et al., 2003;
58Sablowski and Carnier Dornelas, 2014; Tsukaya, 2003). The existence of non-cell
59autonomous control of organ development has been demonstrated in *Arabidopsis*
60leaf (Kawade et al., 2010) but the underlying modes of action remain unclear
61and often system-specific (Ferjani et al., 2007; Han et al., 2014; Horiguchi and
62Tsukaya, 2011; Norman et al., 2011; Okello et al., 2015).

63

64Computational models offer a unique tool to express and test biological
65hypotheses, in a well-defined and controlled manner. Not surprisingly, indeed,
66computational modeling has been largely used to investigate the relationships
67between organ development and the underlying cellular processes. Many works
68have addressed the question of organogenesis, relating local morphogenetic
69rules and cell mechanical properties with the emerging patterns near the
70meristem (Boudon et al., 2015; Dupuy et al., 2010; Kuchen et al., 2012; Löffke
71et al., 2015; Lucas et al., 2013; Robinson et al., 2011) (von Wangenheim et al.,
722016). At the tissue scale, a few models have addressed the issue of cell's size
73variance based on observed kinematic patterns of cell division or growth rates,
74with a particular attention to the intrinsic stochasticity of cell-cycle related
75processes (Asl et al., 2011; Kawade and Tsukaya, 2017; Roeder et al., 2010). In
76most of these models, cell expansion is simply described via an average growth
77rate, possibly modulated by the ploidy level of the cell, without any reference to
78the underlying molecular mechanisms or to the environmental conditions.

79

80 To our knowledge, very few attempts have been made to explicitly model
81the interaction among cell division, expansion and endoreduplication from first
82principles and at the scale of organ development. In Fanwoua et al., 2013 a
83model of tomato fruit development have been developed that integrates cell
84division, expansion and endoreduplication processes based on a set of
85biologically-inspired rules. The fruit is described by a set of q classes of cells with
86the same age, ploidy and mass. Within each class, cell division and
87endoreduplication are described as discrete events that take place within a well-
88defined window of time, whenever a specific mass-to-ploidy threshold is reached.
89Cell growth in dry mass is modeled following a source-sink approach as a
90function of the thermal time, the cell ploidy-level and the external resources. The
91model is able to qualitatively capture the effect of environmental conditions
92(temperature, fruit load) on the final fruit dry mass, but hypotheses and
93parameters are hard to validate as comparaison to experimental data is lacking.
94Moreover, the water content of the cell is not considered preventing the analysis

95of cell volumes.

96 Baldazzi and coworkers developed an integrated model of tomato fruit
97development which explicitly accounts for the dynamics of cell proliferation as
98well as for the mechanisms of cell expansion, in both dry and fresh masses,
99based on biophysical and thermodynamical principles (Baldazzi et al., 2012,
1002013). Here, a new version of this model, which includes cell endoreduplication,
101is proposed. The model was used to investigate different hypotheses concerning
102the regulation and the interaction among cellular processes, with special attention
103to 1) the importance of an organ-wide regulation on cell growth and 2) the
104mechanisms of interaction between endoreduplication and cell expansion
105processes.

106We focus on a natural, wild type organ development and we analyze the effect of
107organ-wide or cell ploidy-dependent regulation onto the dynamics of cell
108expansion. To this aim, different control schemes (either cell-autonomous or
109organ-controlled, with or without ploidy effect on cell expansion) were tested *in*
110*silico* by means of specific model variants. Simulation results were analyzed and
111compared to cell size distributions observed on two contrasted genotypes, a
112cherry and a large-fruited tomato variety.

113The model shows that a pure cell-based control cannot reproduce the observed
114cell size distribution, and an organ-wide control is required in order to get realistic
115cell and fruit sizes. The model also supports the role of endoreduplication as an
116important modulator of the cell expansion potential, although the strength of this
117interaction might be genotype-specific. In particular, results suggest a likely
118interaction through carbon allocation and metabolism.

119

120

121**MATERIALS AND METHODS**

122

123**Experimental data**

124

125Two datasets were collected from two glasshouse experiments performed at

126INRA Avignon (south of France) in 2004 and 2007 on large-fruited (cv Levovil)
127and cherry (cv. Cervil) tomato genotypes of *Solanum lycopersicum* L.
128
129The 2004 experiment fruits were collected from April to May (planting in February)
130whereas in the 2007 experiment fruits were sampled from October to December
131(planting in August). Plants were grown according to standard cultural practices.
132Trusses were pruned in order to homogenise truss size along the stem within
133each genotype. Maximum number of flowers left on each inflorescence was 12
134for Cervil and 6 for Levovil. Flowers were pollinated by bumblebees. Air
135temperature and humidity were recorded hourly in each experiment and input in
136the model as external signals.
137Flower buds and fruits were sampled at different time points relative to the time of
138flower anthesis (full-flower opening). Fruit fresh and dry mass and pericarp fresh
139mass were systematically measured at all times points, before further
140measurements. In 2004, half of the fruit pericarps were then analyzed by flow
141cytometry and the other half were used for the determination of cell number. The
142number of pericarp cells was measured after tissue dissociation according to a
143method adapted from that of Bünger-Kibler and Bangerth, 1982 and detailed in
144(Bertin et al., 2003). Cells were counted in aliquots of the cell suspension under
145an optical microscope, using Fuchs-Rosenthal chambers or Bürker chambers for
146the large and small fruits, respectively. Six to 8 aliquots per fruits were observed
147and the whole pericarp cell number was calculated according to dilution and
148observation volumes. The ploidy was measured in the pericarp tissue, as
149described in Bertin et al., 2007. The average value of three measurements per
150fruit (when allowed for by the fruit's size), was included in the analysis.
151
152In the 2007 experiment, the dynamics of cell number (but not endoreduplication)
153was measured following the same method as in the 2004 experiment. In addition,
154cell size distribution (smallest and largest radii and 2D-surface) distributions were
155measured with ImageJ software (imagej.nih.gov/ij/) in the cell suspension
156aliquots. About 20 to 25 cells per fruit were measured randomly on different
157fruits. Cell size distribution were derived for ripe fruits at about 43 days after

158anthesis (DAA) for Cervil and 60 DAA for Levovil in the considered growing
159conditions.

160

161

162**Model description**

163

164 The model is composed of two interacting modules, both issued from
165previously published models (Bertin et al., 2007; Fishman and Génard, 1998; Liu
166et al., 2007). The fruit is described as a collection of cell populations, each one
167having a specific age, ploidy and volume, which evolve and grow over time during
168fruit development. The number, age (initiation date) and physiological state
169(proliferating or endoreduplicating-expanding cells) of each population is
170predicted by the cell division-endoreduplication module (Bertin et al., 2007),
171based on genotype-specific parameters. It is assumed that the onset of
172endoreduplication coincides with the beginning of the expansion phase, i.e.
173expanding cells are endoreduplicating.

174

175At any time, mass (both fresh and dry component) of expanding cells is computed
176by a biophysical expansion module according to cell's characteristics (age,
177ploidy) and depending on available resources and environmental conditions
178(Fishman and Génard, 1998; Liu et al., 2007). Briefly, cell expansion is described
179by iteratively solving the Lockhart equation relating the rate of volume increase to
180the cell's internal pressure and cell's mechanical properties (Lockhart, 1965).
181Flows of water and solutes across the membrane are described by
182thermodynamic equations and depend on environmental conditions. The relative
183importance of each transport process may vary along fruit developmental stages,
184depending on specific developmental control. A full description of the model and
185its equations can be found in the section S2 of the Supplemental Material.
186In its standard version, the model assumes that all cells have equal access to
187external resources, independently from the number of cells (no competition). All
188the parameters of the division- endoreduplication module are considered to be
189independent from environmental conditions for the time being.

190

191

192**Model initialisation and input**

193

194 The model starts at the end of the division-only phase, when the
195proliferative activity of the cells declines and the expansion phase begins
196(Baldazzi et al., 2013). For Cervil genotype this corresponds to approximately 8
197days before anthesis and to 3 days before anthesis for Levovil genotype (Bertin
198et al., 2007). The initial number of cells, n_0 , was estimated to $1e4$ for the cherry
199tomato (Cervil) and $1.8e5$ for the large-fruited (Levovil) genotype based on a few
200measurements.

201 At the beginning of the simulation, all cells are supposed to be proliferating
202with a ploidy level of 2C (transient ploidy of 4C during cell cycle is not
203considered). Proliferating cells are supposed to have a constant cell mass, m_0 ,
204as often observed in meristematic cells (homogeneous/uniformity in cell size)
205(Sablowski and Carnier Dornelas, 2014; Serrano-Mislata et al., 2015). The initial
206mass of the fruit is therefore $M_f(0)=n_0*m_0=n_0*(w_0 +s_0)$, where w_0 and s_0 are
207initial cell water and dry mass, respectively. At any time, cells leaving the
208proliferative phase start to grow, from an initial mass $2*m_0$ and a ploidy level of
2094C, according to the expansion model and current environmental conditions.

210

211Cell expansion depends on environmental conditions and resources provided by
212the mother plant. The phloem sugar concentration is assumed to vary daily
213between 0.15 and 0.35 M whereas stem water potential oscillates between -0.05
214and -0.6 MPa i.e. typical pre-dawn and minimal stem water potential measured
215for the studied genotypes. Temperature and humidity are provided directly by
216real-time recording of greenhouse climatic conditions.

217

218**Choice of the model variants: control of cell expansion capabilities**

219

220 In the integrated model, a number of time-dependent functions account for
221developmental regulation of cell's metabolism and physical properties during the

222expansion phase (Baldazzi et al. 2013, Liu et al. 2007). Two characteristic time-
223scales are recognizable in the model: the *cell age*, i.e. the time spent since an
224individual cell has left the proliferative phase, and *organ age* i.e. the time spent
225since the beginning of the simulation (Figure 1). Depending on the settings of the
226corresponding time-dependent functions, different cellular processes may be put
227under cell-autonomous or non-cell autonomous control (hereafter indicated as
228organ-wide control), allowing for an *in silico* exploration of alternative control
229hypotheses in the perspective of the cellular and organismal theories. Moreover,
230a direct effect of cell DNA content onto cell expansion capabilities may be tested
231according to biological evidences (Chevalier et al., 2011; Edgar et al., 2014;
232Sugimoto-Shirasu and Roberts, 2003).

233
234As a default all cellular processes are supposed to be dependent on cell age
235(cell-autonomous control) with the only exception of cell transpiration which is
236computed at the organ scale, on the basis of fruit external surface and skin
237conductance, and then distributed back to individual cells, proportionally to their
238relative water content (see section S2).

239
240 Based on literature information and on preliminary tests ((Baldazzi et al.,
2412013, 2017), the switch between symplastic and apoplastic transport, σ_p has
242been selected as the candidate process for an organ-wide control. Indeed,
243intercellular movement of macromolecules across plasmodesmata has been
244shown to be restricted by organ age in tobacco leaves (Crawford and Zambryski,
2452001; Zambryski, 2004) and it is known to be important for cell-to-cell
246communication (Han et al., 2013).

247
248The exact mechanisms by which cell DNA content may affect cell expansion
249remains currently unknown. Based on literature information and common sense,
250three distinct mechanisms of interaction between endoreduplication and cell
251expansion were hypothesized.

252
2531) Endoreduplication has been often associated to an elevated protein synthesis

and transcriptional activity (Chevalier et al., 2014) suggesting a general activation of the nuclear and metabolic machinery of the cell to sustain cell growth (Sugimoto-Shirasu and Roberts, 2003). Following these insights, a first hypothesis assumes an effect of endoreduplication on cell expansion as a ploidy-dependent maximal import rate for carbon uptake. For sake of simplicity, the relation was supposed to be linear in the number of endocycles. The corresponding equation, as a function of the cell DNA content (DNA_c, being 2 for dividing cells, 4 to 512 for endoreduplicating cells), was

262

$$v_m = \langle v^0 \rangle * \log_2(DNA_c) ,$$

264

where $\langle v^0 \rangle$ is the average C uptake activity per unit mass.

266

In addition to a high transcriptional activity, endoreduplicating cells are characterized by a reduced surface-to-volume ratio with respect to 2C cells. As a consequence, it is tempting to speculate that one possible advantage of a high ploidy level may reside in a reduction of carbon demand for cell wall and structural units (Barow, 2006; Pirrello et al., 2018). Such an economy may impact cell expansion capabilities in two ways. First, the metabolic machinery could be redirected towards the synthesis of soluble components, thus contributing to the increase of cell's internal pressure and consequent volume expansion. In the model, the *ssrat* fraction of soluble compound within the cell is

developmentally regulated by the age *t* of the cell (Baldazzi et al. 2013) as

277

$$ssrat = b_{ssrat} (1 - e^{-a_{ssrat} * t}) + ssrat_0$$

279

In the presence of a ploidy effect, the final *bssrat* value was further increased as

281

$$b_{ssrat} = b_{ssrat}^0 * \log_2(DNA_c)$$

283

Alternatively, “exceeding” carbon may be used to increase the rate of cell wall synthesis or related proteins, resulting in an increase of cell wall plasticity (Proseus and Boyer, 2006).

287

In the original expansion model on tomato (Liu et al., 2007) cell wall extensibility

289Phi declines during cell maturation (Proseus et al., 1999) as
290

$$291 \quad \Phi = \Phi_{min} + \frac{(\Phi_{max} - \Phi_{min})}{1 + e^{k(t - t_0)}}$$

292

293In the presence of a ploidy effect, the maximal cell wall extensibility was
294increased as

295

$$296 \quad \Phi_{max} = \Phi_{max}^0 * \log_2(DNAc)$$

297

298

299The individual and combined effects of organ-wide and ploidy-dependent control
300(one process at time) on cell expansion were investigated and compared to a full
301cell-autonomous model. A total of 8 model variants have been tested for each
302genotype, following the complete experimental design shown in Table 1.
303

304**Model calibration**

305Calibration has been performed using genetic algorithm under R software (library
306'genalg'). A two-steps procedure has been used for each tomato genotype.

307First, the division-expansion module (7 parameters) was calibrated on data from
308the 2004 experiment by comparing measured and simulated values of the total
309cells number and the proportion of cells in different ploidy classes, all along fruit
310development. The best fitting parameters were selected and kept fixed for the
311second phase of the calibration, assuming they are independent from
312environmental conditions.

313The expansion module was calibrated on the evolution of pericarp fresh and dry
314mass from the 2007 experiment, for which cell size distribution were measured.
315Six parameters have been selected for calibration based on a previous sensitivity
316analysis (Constantinescu et al., 2016), whereas the others have been fixed to the
317original models' values (Baldazzi et al., 2013; Fishman and Génard, 1998; Liu et
318al., 2007). An additional parameter was estimated for model variants M3 to M7 in
319order to correctly evaluate the strength of the ploidy-dependent control (see

section S3 for more information).

Due to their different structures, the expansion module was calibrated independently for each model variant. The quality of model adjustment was evaluated using a Normalized Root Mean Square Error (NRMSE):

$$NRMSE(x) = 100 \frac{\sqrt{\frac{1}{n} \sum (O_i - S_i(x))^2}}{\frac{1}{n} \sum O_i}$$

where O_i and S_i are respectively, the observed and simulated values of pericarp fresh and dry masses, and n is the number of observations. $x = \{x_1, x_2 \dots x_p\}$ is parameter set of the evaluated solution. The smaller the NRMSE the better the goodness-of-fit is. A NRMSE < 20% is generally considered good, fair if 20% < NRMSE < 30% and poor otherwise.

To avoid high mean fitting errors in each condition, the choice of the best calibration solution was based on a min-max decision criterion (Constantinescu et al., 2016):

$$\min_{x \in X} \left\{ \max \left(NRMSE_{FM}(x), NRMSE_{DM}(x) \right) \right\}$$

where $NRMSE_{FM}$ and $NRMSE_{DM}$ are the normalized root mean square errors for fresh and dry masses respectively. Selected parameters are reported in tables S3 and S4.

Model comparison and selection

On the basis of the best calibration solution, model selection has been performed by comparing measured and simulated cell size distribution, for each model variant. A *semi-quantitative* comparison approach has been used due to the limited experimental information available: the general distribution characteristics (shape, positioning and dispersion) have been characterized rather than a perfect fit. To this aim, 8 descriptive statistical indicators have been computed for each model

345variant and compared to those derived from real- data distribution, namely:

- 346 • skewness and kurtosis (distribution's shape)
- 347 • mean and median cell size (positioning)
- 348 • standard deviation (SD) and median absolute deviation (MAD) (data
- 349 dispersion)
- 350 • maximal and minimal cell size (data dispersion)

351In order to compare different calibration solutions, a *principal component analysis*
352(PCA) was performed on the descriptors of cell distribution arising from each
353model estimation. The ade4 library of R software was used for this purpose (R
354development Core Team 2006).

355

356**RESULTS**

357

358**A characteristic right-tailed distribution of cell areas**

359

360The distribution of cell sizes at a given stage of fruit development directly
361depends on the particular cell division and expansion patterns followed by the
362organ up to the considered time. Any change in the cell division or expansion rate
363will have a consequence on the shape and position of the observed distribution.
364For both tomato genotypes considered in this study, the cell area distribution at
365mature stage shows a typical right-tailed shape (Figure 2), compatible with a
366Weibull or a Gamma distribution (see section S1). The observed cell sizes span
367up to two orders of magnitude, with cell areas (cross section) ranging from 0.004
368to 0.08 mm² for Cervil genotype, and from 0.005 to 0.28 mm² for Levovil (Table
3693 and 4). The average cell area is calculated to be 0.026 mm² for the cherry
370tomato and 0.074 mm² for the large-fruited genotype, values in agreement with
371data from other tomato varieties (Bertin, 2005; Renaudin et al., 2017). Data
372dispersion is higher for the large-fruited genotype, but the shape of the
373distribution, as measured by its skewness and kurtosis values, is pretty similar for
374both tomato varieties.

375

376 Assuming that cell area distribution is a good descriptor of the underlying
377 interaction patterns among cellular processes, a principal component analysis
378 was performed on 8 statistical descriptors of cell size distribution in order to
379 compare predictions of M0-M7 models (see M&M and Tables 2 and 3). For both
380 genotypes, the two first principal components explained approximately 90% of
381 observed variance and were able to correctly separate models with cell-
382 autonomous control (M0, M5, M6) from models including an organ-wide control
383 of cell expansion (Figure 3 and 4). For Cervil genotype, the intra-model
384 variability of cell size distribution was lower than then inter-model differences,
385 whereas for Levovil models including a ploidy-dependent effect resulted in a
386 similar cell size distribution.

387

388 Separation was mainly performed by the first principal component on the basis of
389 the width of the distribution (sd, mad and maximal cell size) and its skewness,
390 both generally increased in models including an interaction between
391 endoreduplication-expansion processes (Figure 3 and 4). Models without organ-
392 wide control are characterized by a reduced dispersion and a larger median
393 value. With respect to experimental data, models combining an organ-wide and a
394 ploidy-dependent control gave the best results.

395

396 In the following, the effect of specific control mechanisms on the resulting cell
397 area distribution is analysed in details, based on the results obtained for the best-
398 fitting solution. The corresponding statistical descriptors are reported in Table 2
399 and 3, for Cervil and Levovil genotype respectively. Note that predicted minimal
400 cell sizes for Levovil genotype are systematically lower than experimental
401 measurements and correspond to the size of proliferating cells. This is partly due
402 to the dissociation method employed for cell counting that underestimates small
403 sub-epidermal cells. These cells although quite numerous contribute little to total
404 pericarp volume and mass (Renaudin et al., 2017).

405

406 **A simple cell-autonomous control scheme leads to unrealistic cell size**
407 **distribution**

408

409 As a benchmark model, the case of a simple cell-autonomous control,
410without ploidy-dependent effect, was first considered (version M0 of the model).
411Accordingly, two cells with the same age, even if initiated at different fruit
412developmental stages, behaved identically in what concerns carbon metabolism,
413transport and wall mechanical properties. In this scheme, therefore, cell size
414variations derived exclusively from the dynamics of cell division, which caused a
415shift in the initiation date for different cohort of cells. When applied to our
416genotypes, the cell-autonomous model was able to reproduce the observed fruit
417mass dynamics but the corresponding cell size distribution was extremely narrow
418(Figure 5 A and D), with standard deviation less than $3e-3$, and strongly left-tailed
419(see Table 2 and 3).

420

421 Including an organ-wide mechanism that controls cell size (model M1)
422introduces a source of variance among cells. In this case, two cells of the same
423age which were initiated at different fruit stages did *not* behave identically,
424resulting in different expansion capabilities and growth patterns (Baldazzi et al.
4252013). Following literature information and preliminary studies (Baldazzi et al.,
4262017) carbon symplastic transport had been supposed under organ-wide control,
427via the progressive closure of plasmodesmata with fruit age (Zambryski,
4282004) (see M&M section). As a result, the cell size distribution got larger, and
429skewness increased towards zero values, indicating a symmetric cell size
430distribution, both for cherry and large-fruited tomatoes. Indeed, the typical right-
431tail observed in experimental data was absent and the maximum cell size
432predicted by the model was much smaller than expected. This suggested that a
433mechanism controlling cell expansion was lacking in the model.

434

435**Endoreduplication and cell growth: possible interactions and genotypic**

436**effect**

437

438A significant correlation between cell size and endoreduplication level has been
439often reported. However, the molecular mechanism by which ploidy could

modulate cell expansion capabilities remain elusive. In this work, three time-dependent cell properties have been selected as possible targets of ploidy-dependent modulation (see M&M section): a) the maximum carbon uptake rate (model version M2), b) carbon allocation between soluble and non-soluble compounds (model version M3) c) cell wall plasticity (model version M4). The three hypotheses were tested independently on both genotypes, in combination with an organ-wide control. Results are shown in Table 2 and 3. In most cases, the addition of a ploidy effect on cell expansion resulted in a positive skewed distribution, with comparable or increased cell size dispersion and maximum cell size with respect to the M0 and M1 models. The strength of the effect however strongly depended on the genotype, with different ranking among model versions.

For Cervil genotype, a potential control of endoreduplication on both the V_{max} (model M2) and cell's allocation strategy (model M3) provided distribution agreement with data, i.e. right-tailed distributions with good dispersion and correct positioning in both mean and median cell area (Figure 5 B and C). The shape of the distribution however was better reproduced by the M2 model, which showed a skewness and kurtosis values close to the observed ones, but the adjustment to fruit growth dynamics, especially for dry mass, was only partially satisfactory (Table 2).

In both models, a high ploidy level resulted in a larger cell size, although the maximum predicted cell size was slightly lower than the observed one. Correlation between ploidy level and cell area was significant with a p-value < 0.0001 (Table S5). The heterogeneity of cell sizes usually observed at each ploidy level was correctly captured by the models as a consequence of the asynchrony in cell division and endoreduplication patterns (Bourdon et al., 2011; Roeder et al., 2010). In comparison to the M2 and M3 models, the potential effect of endoreduplication onto cell's mechanical properties (M4) failed to increase cell size variance beyond the values already obtained without any ploidy effect (M1).

For Levovil genotype, the interaction between endoreduplication and expansion

472 had less impact on the resulting cell distribution. For both model M2 and M4, the
473 addition of a ploidy-dependent effect on cell expansion, although significant, was
474 not able to produce a right-tailed distribution, as observed in experimental data
475 (Figure 5E). The shape of the distribution was pretty symmetric, with skewness
476 values close to zero, reduced dispersion and mean cell size. Only the inclusion of
477 a ploidy-dependent effect on cell's strategy for carbon allocation (model M3)
478 allowed increasing the skewness value up to a reasonable value, but the width of
479 the distribution remained lower than expected with maximal cell sizes not
480 exceeding 0.13 mm² (against the 0.28 mm² observed) (Figure 5F).

481

482

483 **The mechanisms that contribute to cell size variance are likely genotype-** 484 **dependent**

485

486 The above results showed that cell distribution can be significantly affected by
487 both an organ-wide control of fruit development and a direct interaction between
488 endoploidy and cell expansion capabilities. In order to better discriminate their
489 respective role, the effect of a ploidy dependent expansion was tested alone,
490 without the contribution of an organ-wide control of cell growth (models M5-M7).
491 Results confirmed that the best results are obtained by a combined action of both
492 an organ-wide control and a ploidy-dependent modulation of cell expansion but
493 the relative importance of the two mechanisms is likely genotype-dependent.

494

495 In the case of Levovil, organ-wide control turned out to be a major regulatory
496 mode. Independently of the interactions between endoreduplication and
497 expansion, indeed, models without organ-control (models M5-M7) completely
498 failed to reproduce the observations, resulting in a very narrow and left-tailed cell
499 size distribution (Table 3). Organ-wide coordination of cell expansion appeared to
500 be the main responsible for positive skewness of cell size distribution whereas
501 the addition of an endoreduplication-mediated modulation of cell expansion
502 capabilities, alone, resulted only in a marginal improvement of model's
503 performances.

504

505The relative roles of ploidy-dependent and organ-wide control of cell growth
506appear more balanced in cherry tomatoes. Indeed, with the exception of model
507M6, both the organ-wide and the ploidy-mediated control of cell expansion were
508able to reproduce the expected right-tailed distribution shape (Table 2). However,
509their concomitant action was needed in order to get a realistic cell size variance.
510The two mechanisms thus seem to act in synergy to increase cell expansion and
511final cell size.

512

513

514**A combination of ploidy-dependent control of both carbon uptake and**
515**allocation better explains data**

516

517In spite of a good agreement with pericarp data, all tested models failed to fully
518reproduce the observed cell area distribution for Levovil genotype. In particular,
519the maximum reachable cell size predicted by the models was far lower than the
520observed data. Up to now, for seek of simplicity, interaction between
521endoreduplication and expansion has been supposed to affect a single process at
522time but in reality a combination of effects cannot be excluded. We therefore tried
523to combine a ploidy-dependent effect on both the maximum carbon uptake and
524the cell's allocation strategy between soluble and non-soluble compounds (model
525version M23).

526

527Results showed that, for both genotype, the two mechanisms successfully
528combined together to improve cell's expansion capabilities. As a consequence,
529the tail of the distribution straightened and the maximal cell size increased
530towards realistic values (Figure 6). For Cervil genotype, indeed, the predicted cell
531size distribution approached the experimental one, as showed by the projection of
532the M23 model onto the first principal plane (Figure 3). For Levovil genotype, in
533spite of a significant increase of the maximal cell size, the width of the distribution
534remained much lower than in experimental data, resulting into a very long-tailed
535distribution shape.

536

537**DISCUSSION**

538

539 The present paper describes an improved version of an integrated cell
540division-expansion model that explicitly accounts for DNA endoreduplication, an
541important mechanism in tomato fruit development. The model is used to
542investigate the interaction among cell division, endoreduplication and expansion
543processes, in the framework of the neo-cellular theory (Beemster et al., 2003). To
544this aim, 8 model variants including or not an organ-wide control of cell
545development, have been tested and compared to data from two contrasting
546tomato genotypes. Model simulations showed that a pure cellular control was
547unable to reproduce the observed cell size distribution in terms of both average
548cell size and cell variance. In agreement with the neo-cellular theory, the model
549supported the need for an organ-wide control of cell growth, mediated by cell-to-
550cell communication via plasmodesmata (Han et al., 2014; Norman et al., 2011)
551and confirmed the role of endoreduplication as an important modulator of the cell
552expansion potential.

553

554 According to the model, organ-wide control was the main responsible of
555cell-to-cell variance but a ploidy-mediated effect on cell expansion was needed in
556order to obtain large cell surface as the ones observed in experimental data. A
557positive significant correlation between cell size and ploidy level was clearly
558reproduced, independently of model version, in agreement with recent analysis
559of cell size in both leaf epidermis (Kawade and Tsukaya, 2017; Roeder et al.,
5602010) and fruit pericarp (Bourdon et al., 2011).

561

562However, the strength of the correlation may be genotype-dependent. For the
563cherry tomato variety, our modelling approach showed that a ploidy-dependent
564control of carbon metabolism (uptake, allocation or a combination of the two), in
565combination to an organ-wide modulation of cell expansion, was able to generate
566a cell size distribution close to the observed one. For large-fruited tomato
567genotype, interaction between endoreduplication and cell expansion via a single
568process did not suffice to get large cells. Independently of the specific interaction
569mechanism, the model proved unable to correctly reproduce the observed cell

sizes, resulting in a too narrow and symmetric distribution.
The addition of a double effect of cell's endoploidy on both carbon uptake and allocation was able to increase the maximal cell size close to the correct values, but dispersion remained lower than expected.

574

Cell stochasticity may be important to explain cell size distribution in fruit

576

A few reasons may account for the discrepancy observed for the large-fruited variety. A first issue may reside in the quality of experimental data for Levovil genotype. A rapid computation of the average cell size as the pericarp volume over the number of cells at maturity, yields a value significantly lower than the mean of the experimental distribution. This means that the proportion of small cells is underestimated in our dataset, resulting in a distribution that is probably a little flatter and less skewed than the real one.

584

A second, more fundamental reason of discrepancy is rooted in our modelling approach. Our model is an example of population model: the fruit is described a collection of cell groups, each having specific characteristics in terms of number, mass, age and ploidy level, that dynamically evolve during time. Although asynchrony in the emergence of cell groups allowed to capture a considerable part of cell-to-cell heterogeneity, intrinsic stochasticity of cellular processes (Meyer and Roeder, 2014; Robinson et al., 2011b; Smet and Beeckman, 2011) are not accounted for. Variations in the threshold size for division (often associated to a change in the cell cycle duration) as well as asymmetric cell divisions are considered as important determinant of the final cell size (Dupuy et al., 2010; Osella et al., 2014; Roeder et al., 2010; Stukalin et al., 2013) they may contribute to significantly spread the size distribution of both dividing and expanding cell groups, from the early stages. Moreover, the degree of additional dispersion introduced by cell expansion is likely to depend on the specificity of the underlying mechanisms, with possible interactions with ploidy-dependent and organ-wide controls. Of course, these mechanisms are intrinsic of the development of any new organ, independently of the genotype, but they can

602prove more sensitive for Levovil genotype than for Cervil in reason of its higher
603cell number and longer division windows.

604In perspective, the addition of stochastic effects could help to fill the missing
605variance for both Cervil and Levovil genotypes. To this aim, a novel modelling
606scheme is needed in which the average cell mass of a group is replaced by a
607distribution function of cell sizes, whose parameters can evolve with time under
608the effect of cell expansion processes.

609

610**Endoreduplication could regulate cell size through carbon allocation and** 611**metabolism**

612

613 From a biological point of view, the model suggested that
614endoreduplication may interact with cell's carbon metabolism, increasing the
615substrate potentially available for cell expansion. This is in line with literature
616data pointing to ploidy level setting the maximum potential cell size that can be
617attained or not, depending on internal (hormones) and external (environmental)
618factors (Breuer et al., 2010; Chevalier et al., 2011; De Veylder et al., 2011). In
619particular, the model revealed that high ploidy level may increase both the carbon
620uptake rate and its relative allocation to soluble compounds, thanks to an overall
621economy in cell wall synthesis (Barow, 2006; Pirrello et al., 2018).

622

623 It is important to stress that the molecular basis of the supposed interaction
624between endoreduplication and expansion are not described in the model and
625could involve many molecular players. Moreover, the existence of other targets of
626a ploidy-dependent control cannot be excluded nor the contribution of other
627mechanisms to the control of the final cell size. In many fruit species including
628tomato, a negative correlation between average cell size and cell number has
629been observed, suggesting the existence of a competition for resources
630(Lescourret and Génard, 2003; Prudent et al., 2013). This kind of mechanism
631may contribute to broaden the range of attainable cell sizes, increasing size
632variance among first and late-initiated cells (see section S5). The importance of
633such an effect may vary with genotype and environmental conditions (Bertin,

2005; Quilot and Génard, 2008). This may be especially important for large-fruited tomatoes for which cell number is large.

636

Measurement of cell size distribution: promises and challenges to understand the control of fruit growth

639

Further work is needed in order to identify the mechanisms behind organ growth and cell size determination. To this aim, the analysis of cell size distribution shows up as a promising approach. When looking at our results, indeed, the NRMSE with respect to pericarp fresh and dry mass was always between 20% and 30% indicating a satisfactory agreement with data, independently from the model version and the tomato genotype. This highlights the fact that the dynamics of fruit growth alone is not enough to discriminate between several biologically-plausible models. In this sense, cell size distribution represents a much more informative dataset as it uniquely results from the specific cell division and expansion patterns of the organ (Halter et al., 2009).

The assessment of cell sizes in an organ is not an easy task though. As illustrated by the case of Levovil, the employed measurement technique may have important consequences on the resulting cell size distribution (Legland et al., 2012). Indeed, mechanical constraints acting on real tissues as well as vascularisation can largely modify cell shape, resulting in elongated or multi-lobed cells (Ivakov and Persson, 2013). Thus, if the orientation of 2D slices can potentially affect the resulting cell area estimation, possible differences between *in-vivo* tissues and dissociated cells, both in number and size, should also be checked.

The use of mutant or modified strains (Musseau et al., 2017) in combination with recent advancements in microscopy and tomography (Mebatsion et al., 2009; Wuyts et al., 2010) could now permit the acquisition of more reliable datasets, opening the way to an in-depth investigation of cell size variance in relation to their position within the fruit (Renaudin et al., 2017) and to the underlying molecular processes. At term, improvements in the ability of computation models

665to integrate the multiple facets of organ development in a mechanistic way can
666help to evaluate and quantify the contribution of the different processes to the
667control of cell growth.

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669

670**Acknowledgments**

671

672The authors warmly thank B. Brunel for help with cell measurements. The
673authors are grateful to Inria Sophia Antipolis – Méditerranée "NEF" computation
674cluster for providing resources and support. This work was partially funded by the
675Agence Nationale de la Recherche, Project "Frimouss" (grant no. ANR-15-
676CE20-0009) and by the Agropolis Foundation under the reference ID 1403-032
677through the « Investissements d'avenir » programme (Labex Agro:ANR-10-LABX-
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884**Figures**

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887Figure 1: Scheme of the integrated model. The fruit is described as a collection of cell populations,
888each one having a specific age, ploidy and volume. Cells can be either proliferating or expanding-
889endoreduplicating. The number of cells in each class is predicted by the division-
890endoreduplication module, assuming a progressive decline of cells' proliferating activity.
891Expanding cells grow according to the expansion module which provides a biophysical description
892of the main processes involved in carbon and water accumulation. It is assumed that the onset of
893endoreduplication coincides with the beginning of the expansion phase. Two timescales are
894recognizable in the model: the organ age i.e. the time since the beginning of the simulation, and
895the cell age i.e. the time since the cell left the mitotic cycle and entered the expansion-
896endoreduplication phase. Depending on the model version, cell expansion may be modulated by
897organ age (organ-wide control) and/or by cell's ploidy.

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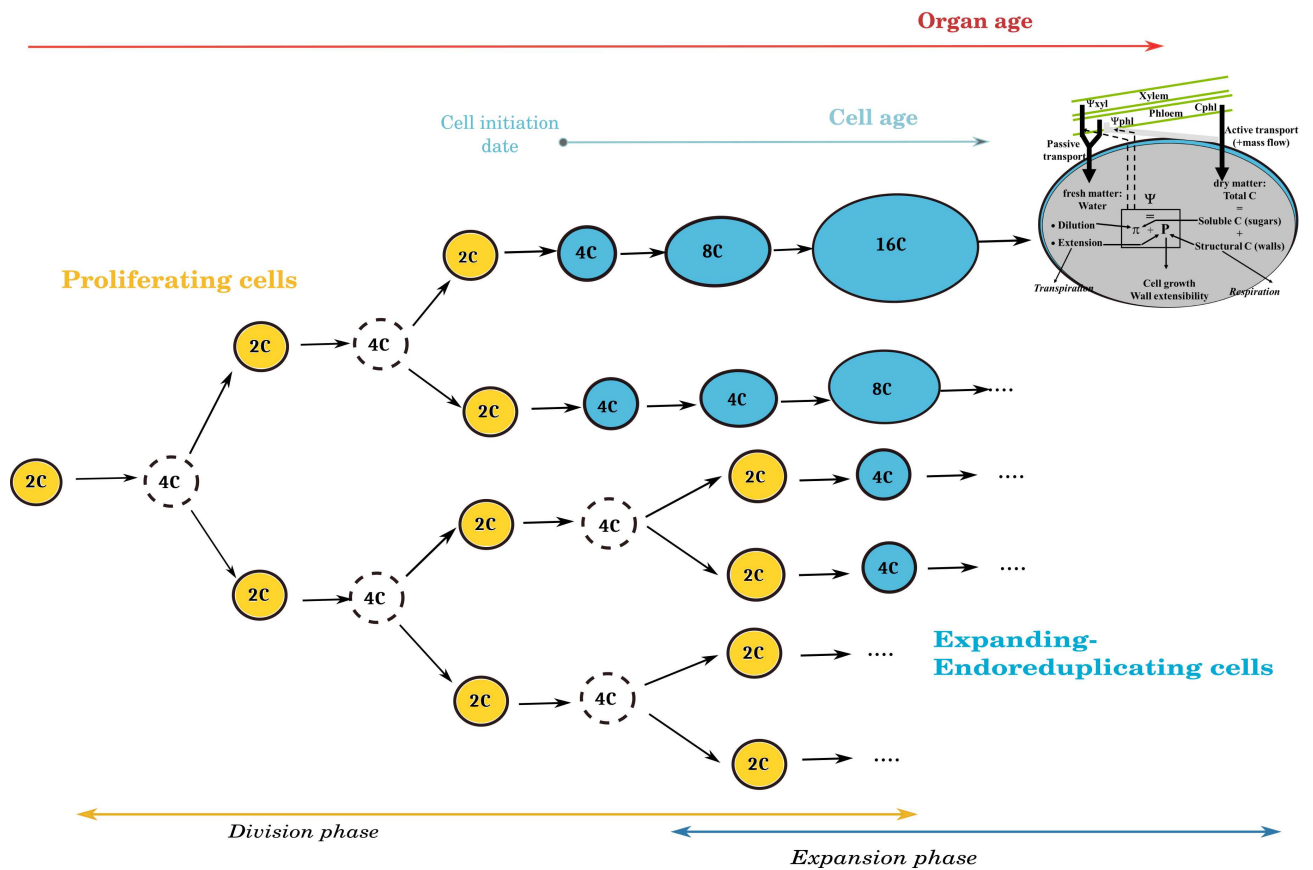
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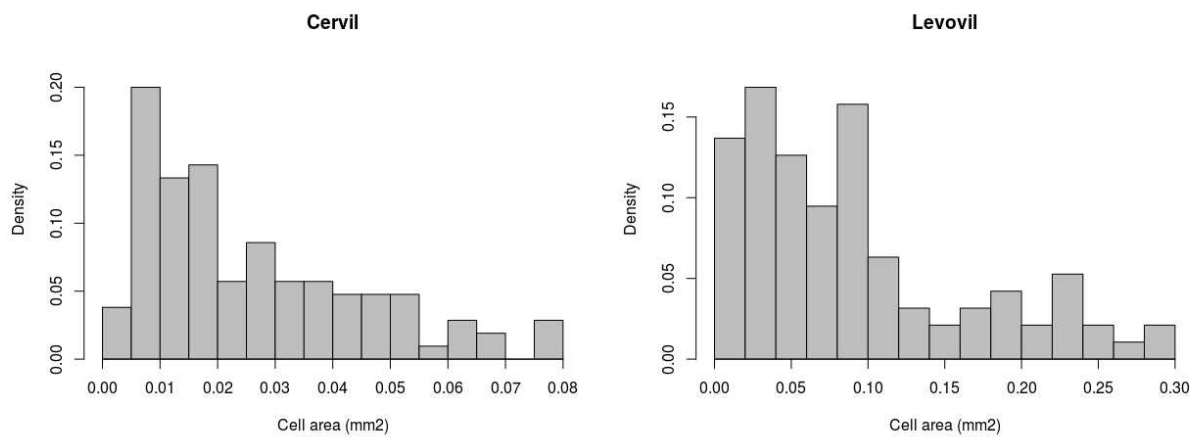
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908Figure 2: Measured cell size distribution at fruit maturity Left: Cervil genotype. Right: Levovil

909genotype.

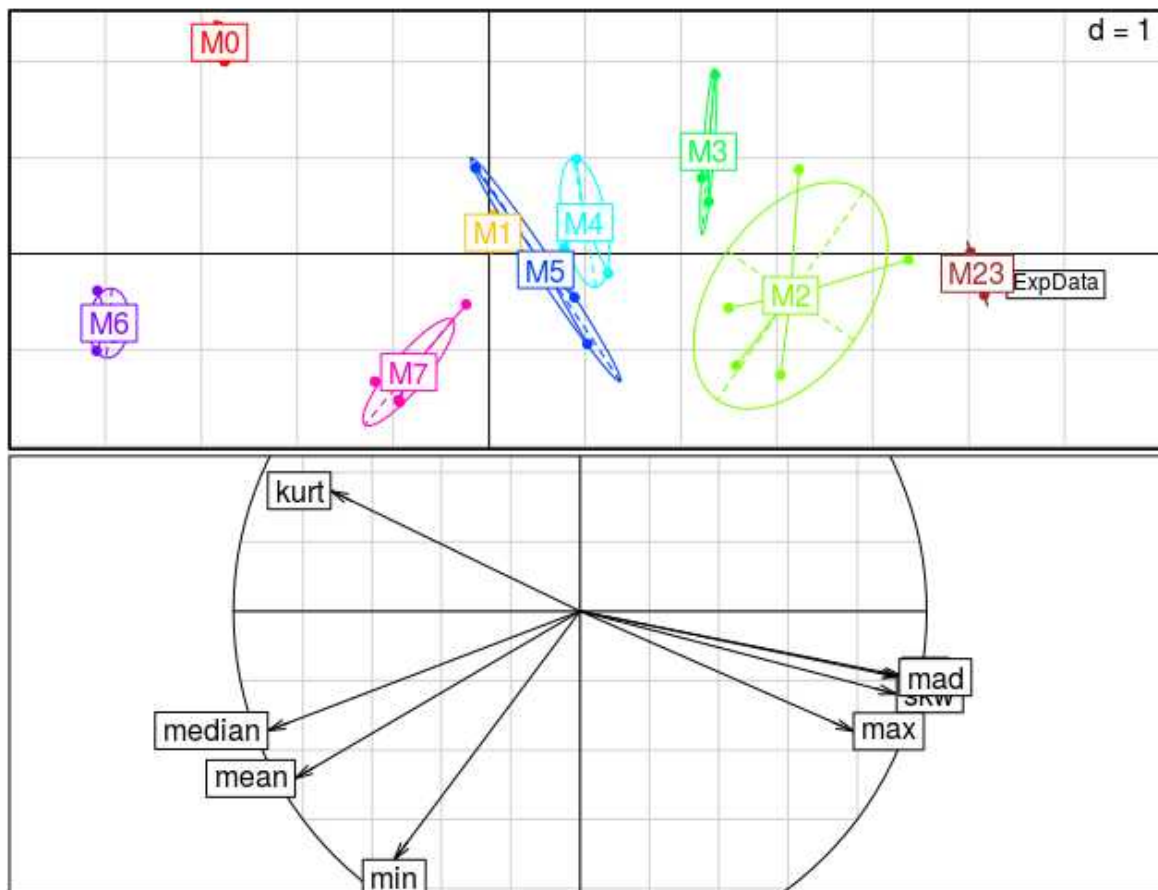
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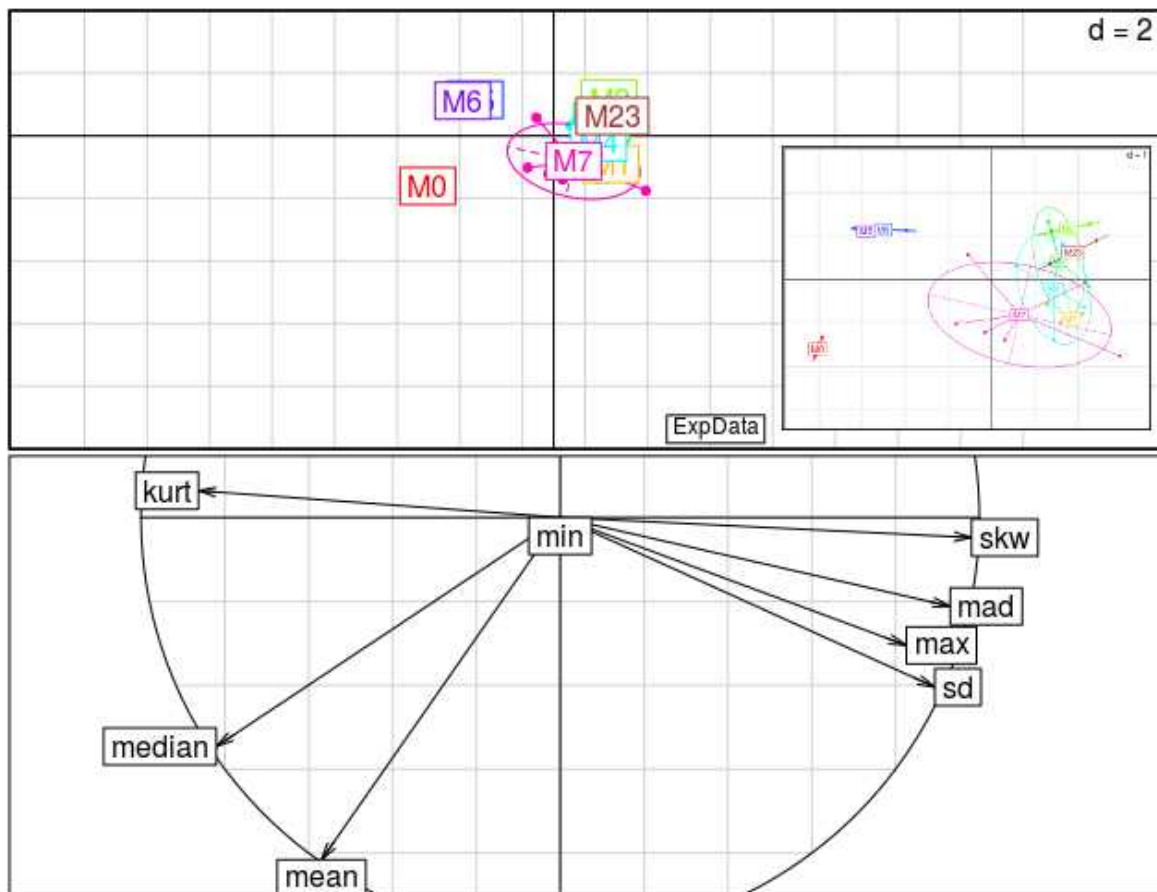
939Figure 3: Principal component analysis (PCA) cell size distributions obtained for the different
 940estimations of models M0-M7 on Cervil genotype. 8 statistical descriptors are used as variables to
 941characterize cell distribution. Measured cell size distribution results of model M23 are projected
 942as a supplementary observation. *Top*: Projection of individual distributions on the PC1-PC2 plane.
 943Model variants are tagged with different colors. *Bottom*: Correlation of the variables with the first
 944two principal components.

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951Figure 4: Principal component analysis (PCA) cell size distributions obtained for the different
 952estimations of models M0-M7 on Levovil genotype. 8 statistical descriptors are used as variables
 953to characterize cell distribution. Measured cell size distribution and results of model M23 are
 954projected as a supplementary observation. *Top*: Projection of individual distributions on the PC1-
 955PC2 plane. Model variants are tagged with different colors. *Bottom*: Correlation of the variables
 956with the first two principal components.

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Figure 5: Predicted cell area distribution at fruit maturity. On the left side, Cervil genotype: A: model M0, B: model M2, C: model M3. On the right side, Levovil genotype: D: model M0, E: model M2, F: model M3.

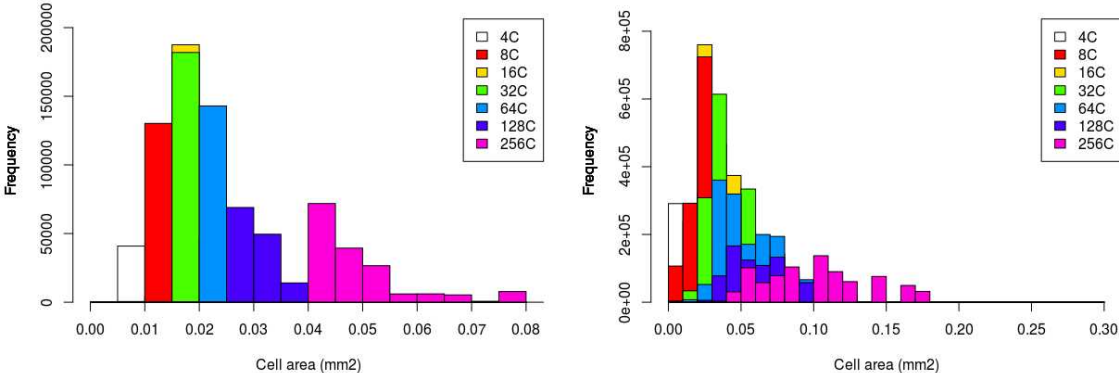
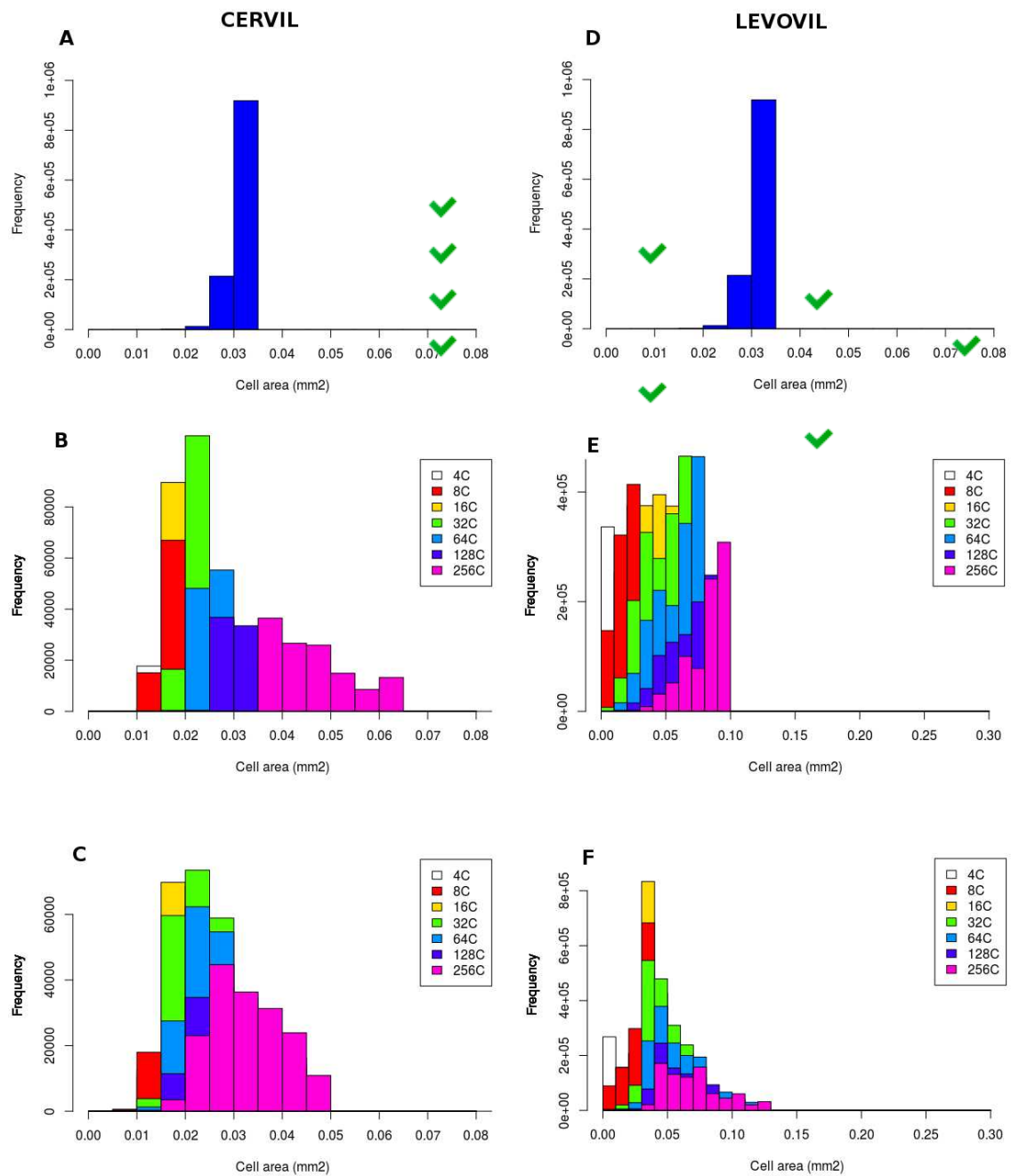


Figure 6: Predicted cell area distribution at fruit maturity for model M23, combining a ploidy-dependent effect on both carbon uptake and allocation. Left: Cervil genotype. Right: Levovil genotype.

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Tables

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 992 Table 1: Experimental design showing the characteristics of the 8 model versions tested
 993 in the paper.

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MODEL	FRUIT MASS		CELL DISTRIBUTION							
	FIT QUALITY		SHAPE		POSITIONING				DISPERSION	
	NRMSE FM	NRMSE DM	Skewness	Kurtosis	Mean	Median	Min	Max	SD	MAD
Exp Data			0.97	3.13	0.026	0.019	0.0039	0.08	0.019	0.016
M0	31.49	31.34	-4.06	30.34	0.030	0.031	0.0042	0.033	0.0015	0.0005
M1	28.32	29.58	0.42	3.07	0.030	0.029	0.0044	0.041	0.0046	0.0042
M2	23	36	0.98	3.58	0.027	0.025	0.0035	0.062	0.011	0.0099
M3	20.18	31.24	0.53	2.58	0.026	0.025	0.0033	0.046	0.0077	0.008
M4	24.97	27.73	0.53	3.45	0.027	0.027	0.0040	0.045	0.005	0.005
M5	34.35	37.02	0.33	3.44	0.029	0.029	0.0040	0.037	0.0035	0.003
M6	34.21	33.81	-3.7	24.9	0.036	0.036	0.0051	0.039	0.002	0.0008
M7	29.53	32.47	0.24	3.34	0.034	0.033	0.0040	0.056	0.005	0.005
M23	23.33	33.05	2.0	8.9	0.026	0.021	0.0037	0.11	0.015	0.009

Table 2 Statistical descriptors for the measured and predicted cell area distribution for Cervil genotype. The NRMSE scores for predicted pericarp dry and fresh masses corresponding to the selected solution are reported under the columns "Fit Quality". For an easier interpretation, green boxes indicate an agreement within 30% with respect to experimental data, yellow an agreement between 30 and 40%, white between 40% and 70% and red a strong discrepancy (over 70% difference with respect to data).

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MODEL	FRUIT MASS		CELL DISTRIBUTION							
	FIT QUALITY		SHAPE		POSITIONING				DISPERSION	
	NRMSE FM	NRMSE DM	Skewness	Kurtosis	Mean	Median	Min	Max	SD	MAD
Exp Data			0.99	3.01	0.092	0.074	0.0048	0.28	0.075	0.063
M0	23.3	25.27	-2.37	7.89	0.069	0.077	0.00049	0.078	0.017	0.0023
M1	25.79	25.32	0.26	2.13	0.059	0.052	0.00049	0.12	0.030	0.034
M2	25.39	25.59	-0.075	2.2	0.047	0.043	0.00049	0.12	0.022	0.017
M3	25.77	25.01	0.67	3.74	0.047	0.043	0.00049	0.13	0.022	0.017
M4	23.64	25.23	-0.15	2.8	0.053	0.054	0.00049	0.11	0.022	0.020
M5	23.36	27.47	-1.51	5.85	0.054	0.056	0.00049	0.077	0.014	0.008
M6	23.61	25.15	-2.22	7.31	0.055	0.060	0.00049	0.066	0.013	0.004
M7	23.74	25.42	-0.56	3.41	0.063	0.066	0.00049	0.10	0.020	0.018
M23	25.45	28.20	1.46	5.73	0.047	0.039	0.00049	0.018	0.028	0.021

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1043Table 3: Statistical descriptors for the measured and predicted cell area distribution for Levovil
1044genotype. The NRMSE scores for predicted pericarp dry and fresh masses corresponding to the
1045selected solution are reported under the columns “Fit Quality”. For an easier interpretation, green
1046boxes indicate an agreement within 30% with respect to experimental data, yellow an agreement
1047between 30 and 40%, white between 40% and 70% and red a strong discrepancy (over 70%
1048difference with respect to data).

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